Health Canada
Pest Management Regulatory Agency

Santé Canada
Agence de réglementation de la lutte antiparasitaire

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PROPOSED REGISTRATION DECISION

Streptomyces lydicus strain WYEC 108

Actinovate SP

(publié aussi en français)

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OVERVIEW

Proposed Registration Decision for Actinovate SP

Health Canada's Pest Management Regulatory Agency (PMRA), under the authority of the *Pest Control Products Act*[1] and Regulations, is proposing full registration for the sale and use of *Streptomyces lydicus* WYEC 108 and the end-use product Actinovate SP, both containing the active ingredient *Streptomyces lydicus* strain WYEC 108, to suppress Botrytis fruit rot and powdery mildew on field and greenhouse strawberries as well as powdery mildew on field and greenhouse peppers and Gerber daisies.

An evaluation of available scientific information found that, under the approved conditions of use, the end-use product has value and does not present an unacceptable risk to human health or the environment.

This Proposed Registration Decision is a consultation document[2] that summarizes the science evaluation of *Streptomyces lydicus* WYEC 108 and Actinovate SP and presents the reasons for the proposed decision. It also proposes additional risk-reduction measures that will be required to further protect human health and the environment.

This overview describes the key points of the evaluation, while the Science Evaluation section provides detailed technical information on human health, environmental and value assessment of *Streptomyces lydicus* WYEC 108 and Actinovate SP.

The PMRA will accept written comments on this proposal up to 45 days from the date of publication of this document. Please forward all comments to Publications (please see contact information indicated on the cover page of this document).

What Does Health Canada Consider When Making a Registration Decision?

The key objective of the *Pest Control Products Act* is to prevent unacceptable risks[3] to people and the environment from the use of pest control products. Health or environmental risk is considered acceptable if there is reasonable certainty that no harm to human health, future generations or the environment will result from use or exposure to the product under its

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1 As per subsection 28(1) of the *Pest Control Products Act*.

2 "Consultation statement" as required by subsection 28(2) of the *Pest Control Products Act.*

3 "Acceptable risks" as defined by subsection 2(2) of the *Pest Control Products Act*.

conditions or proposed conditions of registration. The Act also requires that products have value[4] when used according to the label directions. Conditions of registration may include special precautionary measures on the product label to further reduce risk.

To reach its decisions, the PMRA applies hazard and risk assessment methods as well as policies that are rigorous and modern. These methods consider the unique characteristics of sensitive subpopulations in humans (e.g. children) as well as organisms in the environment (e.g. those most sensitive to environmental contaminants). These methods and policies also consider the nature of the effects observed and the uncertainties present when predicting the impact of pesticides. For more information on how the PMRA regulates pesticides, the assessment process and risk-reduction programs, please visit the PMRA's website at www.pmra-arla.gc.ca.

Before making a registration decision on *S. lydicus* WYEC 108 and Actinovate SP, the PMRA will consider all comments received from the public in response to this consultation document[5]. The PMRA will then publish a Registration Decision document[6] on *S. lydicus* WYEC 108 and Actinovate SP, which will include the decision, the reasons for it, a summary of comments received on the proposed registration decision and the PMRA's response to these comments.

For more details on the information presented in this Overview, please refer to the Science Evaluation section of this consultation document.

What Is Actinovate SP?

Actinovate SP is a biological fungicide containing the bacteria *Streptomyces lydicus* strain WYEC 108 to suppress fungal diseases on field and greenhouse strawberries, peppers and Gerber daisies.

Streptomyces lydicus strain WYEC 108 works by invading and growing within the fungal pathogens, where it releases enzymes that break down the cell wall of fungi. It also readily grows on the tip of plant roots, which protects the plant's roots from plant pathogens by competing with and displacing fungi that may cause disease and by excreting metabolites that target disease fungi.

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4 "Value" as defined by subsection 2(1) of the *Pest Control Products Act*: "...the product's actual or potential contribution to pest management, taking into account its conditions or proposed conditions of registration, and includes the product's (a) efficacy; (b) effect on host organisms in connection with which it is intended to be used; and (c) health, safety and environmental benefits and social and economic impact".

5 "Consultation statement" as required by subsection 28(2) of the *Pest Control Products Act*.

6 "Decision statement" as required by subsection 28(5) of the *Pest Control Products Act*.

❖ Health Considerations

♦ Can Approved Uses of *S. lydicus* strain WYEC 108 Affect Human Health?

***Streptomyces lydicus* strain WYEC 108 is unlikely to affect your health when used according to the label directions.**

People could be exposed to *S. lydicus* strain WYEC 108 when handling and applying the product. The PMRA considers several key factors when assessing health risks: the microorganism's biological properties (e.g. production of toxic byproducts), reports of any adverse incidents, its potential for pathogenicity, infectivity and toxicity as determined in toxicological studies as well as the likely levels to which people may be exposed to this strain relative to exposures already encountered in nature to other strains of the microorganism.

Toxicology studies in laboratory animals describe potential health effects from large doses in hopes of identifying any potential pathogenicity, infectivity and toxicity concerns.

An alternate formulation of Actinovate SP was mildly irritating to the eyes of test animals. Consequently, the signal words "Caution—Skin Irritant" and "May irritate eyes. Avoid contact with eyes" are required on the label.

No other significant toxicity and no signs of pathogenicity or infectivity were observed when *S. lydicus* strain WYEC 108 was tested on laboratory animals.

♦ Residues in Water and Food

Dietary risks from food and water are not of concern.

The *Food and Drugs Act* prohibits the sale of food containing a pesticide residue that exceeds the established maximum residue limit (MRL). Each MRL value determines the maximum concentration in parts per million (ppm) of a pesticide allowed in or on certain foods. Pesticide MRLs are established for the *Food and Drugs Act* purposes through the evaluation of scientific data under the *Pest Control Products Act.* Food containing a pesticide residue that does not exceed the established MRL does not pose an unacceptable health risk.

Streptomyces lydicus strains are common in soil, and application of Actinovate SP to strawberry, pepper and Gerber daisy in fields and commercial greenhouses is not expected to significantly increase the natural environmental background levels of this microorganism. No adverse health effects have been attributed to dietary exposure from natural populations of *S. lydicus*, and none were observed during acute oral toxicity testing. The establishment of a MRL is, therefore, not required for *S. lydicus* strain WYEC 108. Furthermore, the likelihood of residues of *S. lydicus* strain WYEC 108

contaminating drinking water supplies is negligible to non-existent. Consequently, dietary exposure and risk are minimal to non-existent.

♦ Occupational Risks From Handling Actinovate SP

Occupational risks are not of concern when Actinovate SP is used according to the label directions, which include protective measures.

Potential exposure to workers and pesticide handlers from *S. lydicus* strain WYEC 108 is not expected to pose any undue risk. Pesticide applicators handling or applying Actinovate SP and field workers re-entering fields where crops were sprayed, can come into direct contact with *S. lydicus* strain WYEC 108 on the skin, in the eyes or by inhalation. For this reason, the label will specify that applicators and other handlers of Actinovate SP must wear personal protective equipment including waterproof gloves, a long-sleeved shirt, long pants and shoes and socks. Mixer/loaders and applicators must additionally wear a dust/mist filtering mask. Furthermore, early-entry workers will be restricted from entering fields or greenhouses treated with Actinovate SP for up to one hour after spraying or until the solution has dried, unless they are wearing the appropriate personal protective equipment.

Streptomyces lydicus strain WYEC 108 could cause hypersensitivity, especially in people exposed repeatedly to high concentrations of this microbe. The signal words "POTENTIAL SENSITIZER" and precautionary statement "May cause sensitization" are required on the product label to warn workers of this potential hazard. Personal protective equipment and the restricted-entry interval will further mitigate any potential risk to workers and handlers.

For bystanders, exposure is expected to be much less than that of field workers and is considered negligible. Therefore, health risks to bystanders are not of concern.

❖ Environmental Considerations

♦ What Happens When Actinovate SP Is Introduced Into the Environment?

Environmental risks are not of concern.

There are no published reports of disease associated with *S. lydicus* in wild mammals, birds, earthworms, bees and other arthropods, aquatic invertebrates, fish, algae and aquatic plants. Furthermore, a study designed to examine the effects of *S. lydicus* strain WYEC 108 on fish reported no adverse effects. Therefore, *S. lydicus* strain WYEC 108 is expected to present a negligible risk to these non-target organisms.

While there were no reports in published literature of plant disease caused by *S. lydicus*, literature indicated that certain *Streptomyces* species, such as *S. scabies*, *S. acidiscabies* and *S. turgidiscabies* causing "potato scab" on potato, are known plant pathogens. The

applicant referred to growth chamber studies and small-scale field trials conducted with this microbial pest control agent, which demonstrated there were no deleterious effects on potato. Based on the lack of phytotoxicity reported in efficacy trials conducted with Actinovate SP on strawberry, Gerber daisy and bell pepper (see Section 5.2), there is reasonable certainty that no deleterious effects are anticipated on non-target plants.

❖ Value Considerations

♦ What Is the Value of Actinovate SP?

The registration of Actinovate SP would result in an additional non-chemical fungicide for Canadian growers, particularly in the greenhouse industry. It could be used as a resistance management tool because the active ingredient has a multiple-site mode of action. Actinovate SP could also be used as an alternative fungicide in organic fruit production.

Measures to Minimize Risk

Labels of registered pesticide products include specific instructions for use. Directions include risk-reduction measures to protect human and environmental health. These directions must be followed by law.

The PMRA is proposing key risk-reduction measures on the label of Actinovate SP to address the potential risks identified in this assessment.

♦ Human Health

As a standard precaution, anyone handling or applying Actinovate SP must wear waterproof gloves, a long-sleeved shirt, long pants and shoes plus socks. In addition, mixers/loaders and applicators must wear a dust/mist filtering mask. Furthermore, early-entry workers will be restricted from entering fields and greenhouses treated with Actinovate SP for up to one hour after spraying or until solution has dried, unless the appropriate personal protective equipment is worn.

♦ Environment

As a general precaution, handlers are advised not to contaminate irrigation or drinking water or aquatic habitats while cleaning equipment or disposing of waste.

Next Steps

Before making a registration decision on *Streptomyces lydicus* WYEC 108 and Actinovate SP, the PMRA will consider all comments received from the public in response to this consultation document. The PMRA will then publish a Registration Decision document, which will include the decision, the reasons for it, a summary of comments received on the proposed decision and the Agency's response to these comments.

Other Information

At the time the PMRA makes its registration decision, the test data on which the decision is based will be available for public inspection, upon application, in the PMRA's Reading Room (located in Ottawa).

SCIENCE EVALUATION

Streptomyces lydicus strain WYEC 108

1.0 The Active Substance, its Properties and Uses

1.1 Identity of the Active Ingredient

Active micro-organism	*Streptomyces lydicus* strain WYEC 108
Function	Suppression of Botrytis fruit rot (*Botrytis cinerea*) and powdery mildew (*Sphaerotheca macularis*) on field and greenhouse strawberry, suppression of powdery mildew (*Erysiphe cichoracearum*) in field and greenhouse Gerber daisy and for suppression of powdery mildew (*Leveillula taurica*) on field and greenhouse peppers.
Binomial name	*Streptomyces lydicus* strain WYEC 108
Taxonomic designation	
Kingdom	Eubacteria
Phylum	Actinobacteria
Class	Actinobacteria
Order	Actinomycetales
Family	Streptomycetaceae
Genus	*Streptomyces*
Species	*lydicus*
Strain	WYEC 108
Patent Status Information	United States patent Number 5,403,584.
Minimum purity of Active	2.7×10^8 colony forming units (CFU)/g
Identity of relevant impurities of toxicological, environmental and/or significance.	The technical grade active ingredient does not contain any impurities or microcontaminants known to be TSMP Track-1 substances. The product must meet microbiological contaminants release standards and no mammalian toxins are known to be produced by *S. lydicus* strain WYEC 108.

1.2 Physical and Chemical Properties of the Active Ingredients and End-Use Product

Technical Product— *Streptomyces lydicus* WYEC 108

Property	Result
Colour	pale white to cream
Odour	slightly sweet
Physical State	powder
Formulation type	live organism
Guarantee	2.7×10^8 CFU/g
Container material and description	plastic pouches (250 g)
Density	0.2–0.8 g/cc
pH of 1% dispersion in water	7
Oxidizing or reducing action	not corrosive
Storage Stability	Up to 14 months when stored at room temperature (21–26°C)

End-Use Product—Actinovate SP

Property	Result
Colour	pale white to cream
Odour	slight odour of rich soil
Physical state	powder
Formulation type	live organism
Guarantee	1.0×10^7 CFU/g
Container material and description	plastic jars (562 mL[1])
Density	5.5 g/cc
pH of 1% dispersion in water	7
Oxidizing or reducing action	not corrosive
Storage stability	up to 12 months when stored at room temperature (21–26°C)[1]
Flammability	not flammable

[1] Temporary storage stability based on storage stability data from the TGAI

1.3 Directions for Use

Actinovate SP is an end-use product formulated as a powder containing 0.0371% w/w of the microbial pest control agent (MPCA) *S. lydicus* strain WYEC 108 (minimum guarantee of 1.0×10^7 CFU/g) as the sole active ingredient.

Actinovate SP should be applied as a preventative foliar spray. It can be used both in the greenhouse and the field. Application of Actinovate SP at the approved rates at regular intervals throughout the growing season provides effective suppression of Botrytis gray mould and powdery mildew on certain crops. Growers should carefully read and follow label directions, use rates and restrictions.

1.4 Mode of Action

The active ingredient, *S. lydicus* strain WYEC 108 has a multi-site mode of action. The organism produces extracellular chitinase and certain antifungal compounds which affect fungal pathogens.

2.0 Methods of Analysis

2.1 Characterization of the Microorganism and Manufacturing Methods

The data submitted in support of product characterization requirements for *S. lydicus* strain WYEC 108 were sufficient for the accepted use patterns of Actinovate SP. Description of the methods of manufacture and quality assurance of both the *Streptomyces lydicus* WYEC 108 technical product and Actinovate SP end-use product were acceptable and of sufficient detail to support registration.

2.2 Methods for Identification of the Microorganism

Methods to uniquely identify *S. lydicus* strain WYEC 108 are a key component of manufacturing quality assurance.

S. lydicus strain WYEC 108 can be described as spiral-chained, reddish, smooth-surfaced conidia spores. The vegetative mycelium is filamentous without spores. The isolate also has the following characteristics: it does not produce melanin or H_2S pigment when growing on peptone-yeast-iron agar, or peptone-iron agar, it grows between pH 5.6 and 8.0 with an optimal temperature of approximately 30°C (but will grow at 37°C) and white colonies initially form aerial mycelia which become gray. *S. lydicus* strain WYEC 108 is similar in biochemical characteristics to ATCC 25740 (*S. lydicus*), with a slight difference in sporulation (– or +) with glucose, sucrose, galactose, inositol, xylitol, arabinose and sodium pyruvate.

S. lydicus strain WYEC 108 can be identified from within natural actinomycete populations based on its antibiotic resistance profile. Viable plate counts on a selective media amended with carbenicillin (200 µg/mL), spectinomycin (10 µg/mL), streptomycin (10 µg/mL), neomycin (10 µg/mL), ampicillin (100 µg/mL) and cyclohexamide (100 µg/mL) have positively identified the MPCA from among soil microorganisms since most soil bacteria, including actinomycetes, are sensitive to these antibiotics.

Randomly amplified polymorphic DNA (RAPD) techniques were used to develop a strain specific probe to specifically identify *S. lydicus* strain WYEC 108 from other Streptomycetes. A RAPD fingerprint profile was generated for genomic DNA of *S. lydicus* strain WYEC 108 and clearly distinguishable products between 0.2 and 1.2 kDa were selected and radio-labelled. The radio-labelled probes were used to screen RAPD fingerprint blots of other *Streptomyces* and non-*Streptomyces* to determine the specificity of the probes. An intense signal at 0.3 kDa, with a less intense 0.8-kDa band which were specific to *S. lydicus* strain WYEC 108 were visualized by Southern blotting. The 0.3-kDa probe has been sequenced and assigned GenBank number AF239669. This probe can be used for strain-specific identification of the MPCA using polymerase chain reaction (PCR).

2.3 Methods to Define the Content of the Microorganism in the Manufactured Material Used for the Production of Formulated Products

The potency (CFU/mL) of the technical grade active ingredient (TGAI) is routinely checked throughout the manufacturing process using the plate count method on sporulation agar. Serial dilutions of spore preparations are plated and incubated and then counted for colony formation.

2.4 Methods for Determination of Relevant Impurities in the Manufactured Material

The quality assurance procedures used to limit contaminating microorganisms during manufacture of Actinovate SP are acceptable. If any contamination is discovered the contaminated lot is autoclaved and discarded.

There are no impurities of toxicological concern associated with *Streptomyces lydicus* WYEC 108 and based on the processing and manufacturing methods the possibility of contamination and introduction of unintentional ingredients are minimal.

There are no reports of mammalian toxins produced by this MPCA. However, Streptomycetes are known for their production of biologically active secondary metabolites, some of which can cause genotoxic effects. There were no reports in published literature of genotoxic metabolites produced by *S. lydicus* and there is no evidence to suggest that *S. lydicus* strain WYEC 108 would produce any genotoxic compound.

2.5 Methods to Determine Storage Stability, Shelf-life of the Microorganism

Storage stability data for the technical grade active ingredient included two lots manufactured in 1999 stored at 21–26°C for 14 months and five lots manufactured in 2003 stored at 21–26°C for 14 months. Data showed that the technical grade active ingredient is stable for this duration under the conditions specified.

Storage stability data for the end-use product was not provided. The storage stability data on the TGAI is considered adequate to tentatively support registration of the end-use product for a period of up to 12 months.

However, storage stability data for the end-use product will be required as a condition of registration. The data must confirm the viability of the technical grade active ingredient in the end-use product on the day of manufacture and at subsequent time points thereafter.

3.0 Impact on Human and Animal Health

3.1 Toxicity and Infectivity Summary

The PMRA conducted a detailed review of the mammalian toxicological database for *S. lydicus* strain WYEC 108. The database is largely complete, consisting of laboratory animal (in vivo) toxicity studies (acute oral toxicity, acute pulmonary toxicity/pathogenicity and infectivity, intravenous injection infectivity, primary eye irritation and dermal irritation) currently required for health hazard assessment purposes which were carried out in accordance with currently accepted international testing protocols and Good Laboratory Practices. A waiver request was deemed acceptable to address the acute dermal toxicity of Actinovate SP in lieu of testing. The scientific quality of the data is high and the database is considered sufficient to characterize the toxicity and infectivity of this pest control agent and associated end-use product. In the acute pulmonary toxicity study and the intravenous infectivity study, the test substance was identical to the technical grade active ingredient proposed for registration. In the acute oral toxicity study, the eye irritation study and the dermal irritation study, the test substance was “WYEC 108-EP”. The toxicity studies using WYEC 108-EP as the test substance are acceptable for assessing the toxicity potential of Actinovate SP.

In the acute oral toxicity study no mortalities and no significant toxicity other than minor clinical signs on Day 1 (soft faeces/very slight diarrhea, slight piloerection) were observed in Sprague-Dawley rats following oral gavage with 5.4×10^8 CFU of *S. lydicus* strain WYEC 108 per animal. Infectivity was not assessed.

In the pulmonary toxicity/pathogenicity and infectivity study, no mortalities were observed in Sprague-Dawley rats following intratracheal instillation at doses ranging from 10^6 to 10^8 CFU of *S. lydicus* strain WYEC 108 per animal. No significant signs of toxicity were observed other than on Day 1 where one female test animal was observed with slight piloerection, raspy

breathing, dark crust on eyes and walking on tiptoes, as well as minor transient weight losses in some animals from the treatment and the heat-killed test substance control groups. The MPCA was detected in the kidney, liver, mesenteric lymph nodes and in the lungs of treatment test animals up to Day 21, but had cleared by Day 28 or earlier.

In the intravenous injection infectivity study, no mortalities or significant signs of toxicity were observed in Sprague-Dawley rats following injection with *S. lydicus* strain WYEC 108 at 9.33×10^8 CFU per animal. *S. lydicus* strain WYEC 108 was detected in the blood, brain, kidney, mesenteric lymph nodes, lungs, liver and spleen up to Day 21, but had cleared from all test animals by Day 28 or earlier.

In the eye irritation study, slight opacity of the cornea, with some swelling of the conjunctivae, was observed in the eyes of animals dosed with 0.1g of WYEC 108-EP, but it was resolved or reduced by 24 hours. All signs of irritation were resolved within 72 hours and the end-use product was classified as mildly irritating. The signal words "CAUTION: EYE IRRITANT" and precautionary statements instructing users to avoid contact with eyes are required on the *Streptomyces lydicus* WYEC 108 and Actinovate SP label.

In the acute dermal irritation study, only very slight erythema was observed in one test rabbit following dermal exposure to 0.5 g of WYEC 108-EP (containing 5.0×10^8 CFU *S. lydicus* strain WYEC 108).

A waiver request for an acute dermal toxicity/pathogenicity study with the Actinovate SP product was submitted in lieu of testing. The request was based on the results from the acceptable acute toxicity, acute pulmonary toxicity/pathogenicity, intravenous injection infectivity, primary eye irritation and primary dermal irritation studies that demonstrated the lack of toxicity, pathogenicity, infectivity and mild eye irritation for the active ingredient *S. lydicus* strain WYEC 108. As such, the data waiver request was found to be acceptable to fully assess the risks associated with the MPCA given the intended use pattern of Actinovate SP.

Although the applicant has reported that there have been no incidents of hypersensitivity during product development, production and testing of *S. lydicus* strain WYEC 108 or Actinovate SP, like all microbial agents, *S. lydicus* strain WYEC 108 is considered a potential sensitizing agent. The signal words "POTENTIAL SENSITIZER" and label statements indicating that *S. lydicus* strain WYEC 108 and Actinovate SP may cause sensitization and requirements for personal protective equipment (PPE), including a dust/mist filtering respirator meeting NIOSH standards of at least N–95, R–95 or P–95 and judicious handling to minimize exposure of workers are required.

The additional need for higher tier subchronic and chronic toxicity studies were not required because of the low acute toxicity of the MPCA and the lack of indications of infectivity, toxicity or pathogenicity in the test animals treated in the Tier I acute pulmonary toxicity/infectivity and intravenous injection infectivity studies.

A survey of published literature indicated that, in general, *Streptomyces* species are known for their production of antimicrobial substances and are not often the cause of infection. While there were no clinical cases involving *S. lydicus* in literature, clinical cases involving other *Streptomyces* species were reported. The more prominent reports include cases of mycetoma, a chronic subcutaneous infection in humans caused by *S. somaliensis*. Certain thermophylic actinomycetes, such as *S. thermohygroscopicus* and *S. albus*, have also been related to the development of an immunological disease known as allergic alveolitis. Allergic alveolitis, also known as Farmer's Lung or hypersensitivity pneumonitis, is an occupational disease of grain farmers that is characterized by lesions of the lung. Other rare cases of *Streptomyces* infections (brain abcesses, bacteremia, endcarditis, eye infection and chronic pericarditis) have also been reported.

Within the available scientific literature, there are no reports that suggest *S. lydicus* has the potential to cause adverse effects on the endocrine system of animals. The submitted toxicity/infectivity studies in the rodent indicate that, following the pulmonary and intravenous routes of exposure, the immune system is still intact and able to process and clear the MPCA. Based on the weight of evidence of available data, no adverse effects to the endocrine or immune systems are anticipated for *S. lydicus* strain WYEC 108.

3.2 Occupational and Bystander Exposure and Risk Assessment

3.2.1 Occupational

When handled according to the label instructions, the potential routes of handler exposure to *S. lydicus* strain WYEC 108 are pulmonary, dermal and to some extent, ocular.

Since unbroken skin is a natural barrier to microbial invasion of the human body, dermal absorption could occur only if the skin were cut, if the microbe were a pathogen equipped with mechanisms for entry through or infection of the skin, or if metabolites were produced that could be dermally absorbed. *S. lydicus* has not been identified as a wound pathogen and there is no indication that it could penetrate intact skin of healthy individuals. Based on the results of the dermal irritation study, the review of published literature and the formulation ingredients in Actinovate SP, the PMRA expects that dermal irritation from Actinovate SP will be minimal and that the potential for dermal toxicity is low.

The PMRA assumes that all microorganisms contain substances that can elicit positive hypersensitivity reactions and respiratory hypersensitivity could be expected to develop upon repeated exposure to microbial pest control products (MPCPs). Therefore all MPCAs are considered potential sensitizers. Label requirements for personal protective equipment and risk mitigation measures, such as a restricted-entry interval, are required to protect populations that are likely to be primarily exposed to the pesticide. Such exposure to mixer/loaders, applicators and early-entry workers can be minimized if they wear gloves, long-sleeved shirts, long pants, shoes and socks and a dust/mist filtering respirator meeting NIOSH standards of at least N–95,

R–95 or P–95. A restricted-entry interval (REI) of 1 hour or until the solution has dried will also minimize potential exposure. The signal words “POTENTIAL SENSITIZER” and precautionary statements that the products may cause sensitization are required on the *S. lydicus* strain WYEC 108 and Actinovate SP labels.

An eye irritation study conducted with the undiluted test substance, WYEC 108-EP, classified the product as mildly irritating to the eyes. To protect populations that are likely to be primarily exposed to the product, the signal words “CAUTION: EYE IRRITANT” and precautionary statements aimed at instructing workers to avoid contact with the eyes will be imposed on the technical grade active ingredient and end-use product labels.

3.2.2 Bystander

Overall the PMRA does not expect that bystander exposure will pose an undue risk on the basis of the low toxicity/pathogenicity and infectivity profile of *S. lydicus* strain WYEC 108 and the assumption that precautionary label statements will be followed in the use of Actinovate SP.

The end-use product label does not allow applications to turf, residential or recreational areas therefore non-occupational dermal exposure and risk to adults, infants and children are expected to be low. As the use sites are agricultural, exposure to infants and children in school, residential and daycare facilities is likely to be minimal to non-existent. Consequently, the health risk to infants and children is expected to be negligible.

3.3 Dietary Exposure and Risk Assessment

3.3.1 Food

While the proposed use pattern may result in some dietary exposure with possible residues in or on agricultural commodities, negligible to no risk is expected for the general population, including infants and children because *S. lydicus* strain WYEC 108 did not demonstrate oral toxicity at the maximum dose tested in the Tier I acute oral toxicity study. A Tier I acute pulmonary toxicity/pathogenicity and infectivity study and an intravenous injection infectivity study also demonstrated no toxicity, pathogenicity or infectivity at the maximum dose tested. As such, higher tier subchronic and chronic dietary exposure studies were not required. Therefore, there is no concern for chronic risks posed by dietary exposure of the general population and sensitive subpopulations, such as infants and children.

3.3.2 Drinking Water

Although *S. lydicus* strain WYEC 108 could enter neighbouring aquatic environments via spray drift or surface-water runoff, no risks are expected from exposure to this microorganism via drinking water because exposure will be minimal and because *S. lydicus* strain WYEC 108 showed no harmful effects in laboratory animals that were exposed orally in Tier I acute oral toxicity testing. Specific product labelling will be required to limit spray drift and surface water runoff. The potential for transfer of *S. lydicus* strain WYEC 108 to surface or ground water during run-off is considered minimal to non-existent due in part to its percolation through and

resulting capture in soil, where the organism can be found naturally. The Actinovate SP label instructs users not to allow the product to enter bodies of water during use or disposal. Furthermore, municipal treatment of drinking water will reduce the transfer of residues to drinking water. Therefore, potential exposure to *S. lydicus* strain WYEC 108 in surface and drinking water is negligible.

3.3.3 Acute and Chronic Dietary Risks for Sensitive Subpopulations

Calculation of acute reference doses (ARfDs) and acceptable daily intakes (ADIs) is not usually possible for predicting acute and long term effects of microbial agents in the general population or to potentially sensitive subpopulations, particularly infants and children. The single (maximum hazard) dose approach to testing MPCAs is sufficient for conducting a reasonable general assessment of risk if no significant adverse effects (i.e., no acute toxicity, infectivity or pathogenicity endpoints of concern) are noted in acute toxicity and infectivity tests. Based on all the available information and hazard data, the PMRA concludes that *S. lydicus* strain WYEC 108 is of low toxicity, is not pathogenic or infective to mammals and that infants and children are likely to be no more sensitive to the MPCA than the general population. Thus there are no threshold effects of concern and, as a result, no need to require definitive (multiple dose) testing or apply uncertainty factors to account for intra- and interspecies variability, safety factors or margins of exposure. Further, factoring in consumption patterns among infants and children, special susceptibility in these subpopulations to the effects of this MPCA (i.e. neurological effects from pre- or post-natal exposures) and cumulative effects of the MPCA and other registered microorganisms that have a common mechanism of toxicity on infants and children, do not apply to *S. lydicus* strain WYEC 108. As a result, the PMRA has not used a margin of exposure (safety) approach to assess the risks of *S. lydicus* strain WYEC 108 to human health.

3.4 Maximum Residue Limits

The *Food and Drugs Act* (FDA) prohibits the sale of adulterated food, that is, food containing a pesticide residue that exceeds the established maximum residue limit (MRL). Pesticide MRLs are established for FDA purposes through the evaluation of scientific data under the *Pest Control Products Act* (PCPA). Each MRL value defines the maximum concentration in parts per million (ppm) of a pesticide allowed in/on certain foods. Food containing a pesticide residue that does not exceed the established MRL does not pose an unacceptable health risk.

As a product containing a naturally occurring soil microorganism, the use of Actinovate SP is not expected to significantly increase the natural environmental background levels of this microorganism. No adverse effects from dietary exposure have been attributed to natural populations of *S. lydicus*. Furthermore, no adverse effects were observed in the acute oral toxicity study and there are no reports of known mammalian toxins being produced by the MPCA. The establishment of MRLs is, therefore, not required for *S. lydicus* strain WYEC 108 under Section 4(d) of the *Food and Drugs Act* (adulteration of food) as defined under Division 15, Section B.15.002 of the Food and Drugs Regulation.

3.5 Aggregate Exposure

Based on the toxicity/pathogenicity and infectivity test data submitted and other relevant information in the PMRA's files, there is reasonable certainty no harm will result from aggregate exposure of residues of *S. lydicus* strain WYEC 108, to the general Canadian population, including infants and children, when the MPCP is used as labelled. This includes all anticipated dietary (food and drinking water) exposures and all other non-occupational exposures (dermal and inhalation) for which there is reliable information.

Since the product is to be applied to outdoor agricultural sites and is not allowed for use on turf, residential or recreational areas, dermal and inhalation exposure to the general public will be very low. Furthermore, no significant clinical signs were observed in laboratory animals exposed orally or by pulmonary instillation to *S. lydicus* strain WYEC 108 at maximum hazard doses and there are no adverse effects reported from exposure to other strains of *S. lydicus* encountered in the environment. Even if there is an increase in exposure to this microorganism from the use of Actinovate SP, it is anticipated that there will be no increase in potential human health risk.

3.6 Cumulative Effects

The PMRA has considered available information on the cumulative effects of residues and other substances that have a common mechanism of toxicity on the total population as well as infant and children. Besides naturally occurring strains of *S. lydicus* in the environment, the PMRA is not aware of any other microorganisms or substances that share a common mechanism of toxicity with this active ingredient. No cumulative effects are anticipated if the residues of *S. lydicus* strain WYEC 108 interact with related strains of this microbial species.

4.0 Impact on the Environment

4.1 Fate and Behaviour in the Environment

Environmental fate testing is intended to demonstrate whether an MPCA is capable of surviving or replicating in the environment to which it is applied and provide an indication of which non-target organisms may be exposed to the MPCA, as well as provide an indication of the extent of exposure. Environmental fate data (Tier II/III) are not required due to the absence of significant toxicological effects in non-target organisms in Tier I testing. No studies were submitted to address the environmental fate and behaviour of *S. lydicus* strain WYEC 108. There is however, information on the environmental fate of *S. lydicus* available in the published literature.

The ubiquitous nature of *S. lydicus* as a soil microorganism suggests that it will survive under field conditions. Typically, the level of spores of a given *Streptomyces* species in a rich organic soil will average 10^3 or 10^4 per gram of dry soil. When growth conditions are optimal, vegetative cells, reported as colony forming units per gram of dry soil (CFU/g) may rise to 10^7 to 10^8 CFU/g of dry soil. Most of the time *S. lydicus* are dormant spores in the soil and periods of growth are usually short-lived (e.g. a few hours to days in between months of dormancy).

Published studies indicate that *S. lydicus* can colonize the root system of many plants where it acts as a naturally occurring plant growth-promoting bacterium. Like many *Streptomyces* species, *S. lydicus* is quantitatively and qualitatively important in the rhizosphere of host plants where it influences plant growth and protects plant roots against invasion by root pathogenic fungi. Beneficial effects from *S. lydicus* in association with chickpea, pea, carrot and beets have been reported.

Although typically characterized as soil bacteria, *Streptomyces* species have been isolated from water and sediment samples from freshwater lakes and rivers. While *Streptomyces* species have been isolated from mud samples taken from the English Lake District of the United Kingdom, the inability of the isolates to grow in native and enriched mud in vitro suggests that the organisms are relatively inactive in that environment. Metabolically active *Streptomyces* species have, however, been isolated from freshwater streams and fish ponds in Denmark. *Streptomyces* species have also been isolated from submersed aquatic plants (*Sparganium americanum, Ranunculus pusillus* and *Microanthemum umbrosum*) from freshwater streams in North Carolina (USA) using various selective measures for Actinomycetes.

Streptomyces species have also been isolated from water and sediment samples from estuarine and marine environments. To investigate the ecological significance of these findings ribosomal ribonucleic acid (rRNA) extracted from coastal marsh sediments were hybridized with a *Streptomyces*-specific gene probe. The findings demonstrated that *Streptomyces* rRNA accounted for 2 to 5% of the sediment community rRNA and that the source of the hybridization signal could not have been spores. *Streptomyces* species have also been isolated from marine plants such as sea algae (*Ulva lactura* and *U. Enteromorpha*) and macro-algae (*Gracilaria verrucosa*) and from marine animals such as sea anemone (*Actiniaria* sp.), ground shark (*Mustelus manazo*), sea hare (*Apylysia dactylomela*) and marine sponge (*Craniella australiensis*).

In a submitted acute toxicity study on freshwater fish (see Section 4.2.2), rainbow trout (*Oncorhynchus mykiss*) were exposed to *S. lydicus* strain WYEC 108 in the water at a series of concentrations ranging from 3.0×10^3 to 6.3×10^4 CFU/mL (mean measured) for 96 hours. However, viable plate counts of water samples collected throughout the test showed a decline in viable numbers in all test solutions to as low as 8.8% of the original measured concentration after 96 hours. These results would suggest that *S. lydicus* strain WYEC 108 is not well-adapted to survival in fresh water.

4.2 Effects on Non-Target Species

4.2.1 Effects on Terrestrial Organisms

No studies were submitted to address the effects of Actinovate SP on terrestrial organisms. Therefore, the potential risk of *S. lydicus* strain WYEC 108 to terrestrial organisms was assessed based on reports in the published literature on other strains of *S. lydicus* and related *Streptomyces* species, as well as information gathered under Part 3.0 Impact on Human and Animal Health.

For terrestrial vertebrates, no reports of adverse effects from *S. lydicus* in wild mammals or bird populations were found in the published literature. Since *S. lydicus* is ubiquitous in the environment, wild mammals and bird populations are considered to have been exposed to indigenous populations of the organism with no incidents of adverse effects reported. The laboratory animal studies on the rat submitted in support of this registration and reviewed in Section 3.1 indicate that there is no toxicity or pathogenicity to rodents from testing at maximum hazard dose levels. Based on these results, toxic or pathogenic effects from the MPCA in wild mammals or birds are not anticipated.

For terrestrial arthropods (including honeybees), there were no cases of adverse effects from *S. lydicus* to insects, including honeybees in published literature. Since *S. lydicus* is ubiquitous in the environment, terrestrial arthropods are considered to have been exposed to indigenous populations of the organism with no incidents of adverse effects reported. Published literature did reveal that other *Streptomyces* species have shown effects on lepidopteran insect species by the production of various secondary metabolites, such as avermectin, macrotetrolides and virginiamycins. This is not surprising given that *Streptomyces* species are known for their production of a vast array of biologically active compounds. There is no indication that the MPCA produces any of these insecticidal compounds.

Symbiotic interactions between *Streptomyces* species and the European beewolf (*Philanthus triangulum*, Hymenoptera, Crabronidae) have also been described in literature. The bacteria, which are located in the antennal glands of females, appear to protect the wasp offspring against pathogens. *Streptomyces* species have also been isolated from the termite (*Termitidae* sp.) gut as an apparent constituent of the normal gut microflora.

For earthworms and other soil macroorganisms, no studies were submitted to address the effects of Actinovate SP on earthworms or other nonarthropod invertebrates. Effects data for soil macroorganisms are not required because the product is not intended to control pest nonarthropod invertebrates or soil macroorganisms and the proposed use patterns are not likely to considerably increase natural populations of *S. lydicus* in the soil.

Nevertheless, published literature indicated that certain *Streptomyces* species have demonstrated toxicity to certain nematode species. The effects were most often related to the production of various secondary metabolites. This is not surprising given that *Streptomyces* species are known for their production of a vast array of biologically active compounds. Species of nematodes for which effects from *Streptomyces* species have been reported include non-parasitic roundworms (*Caenorhabditis elegans*), parasitic reniform nematode (*Rotylenchulus reniformis*), soybean cyst nematode (*Heterodera glycines*), parasitic cotton-root knot nematode (*Meloidogyne incognita*), army worm (*Pseudaletia unipuncta*), southern army worm (*Spodoptera eridania*) and pine wood nematode (*Bursaphelenchus lignicolus*). Other *Streptomyces* species have shown to inhibit larval development in parasitic nematode *Haemonchus contortus* and reduce egg hatch and motility in plant parasitic nematode *Heterodera glycines*. There is no evidence to suggest that the MPCA produces any nematocidal compounds.

No study was submitted to address the effects of Actinovate SP on other soil microorganisms. Although the product is intended to control pest microorganisms, effects data are not required since *S. lydicus* is a normal component of the soil and *S. lydicus* strain WYEC 108 is not expected to affect environmentally or economically important microbial species or microbiologically-mediated biogeochemical processes.

For terrestrial plants, published literature indicated that *S. lydicus* is ubiquitous in the environment and acts as a naturally occurring plant growth-promoting bacterium in the rhizosphere of various host plants. Beneficial effects from *S. lydicus* in association have been reported for chickpea, pea, carrot and beets.

However, certain *Streptomyces* species are also known plant pathogens. Among the most phytopathogenic are *S. scabies*, *S. acidiscabies* and *S. turgidiscabies* which cause a severe, necrotic disease of potato known as "potato scab". A single report in published literature also identified an *S. lydicus* isolate from a potato scab lesion. There were no other reports relating *S. lydicus* to potato scab disease and all other published studies indicate that *S. scabies*, *S. acidiscabies* and *S. turgidiscabies* are much more often associated with potato scab disease. Furthermore, the registrant indicated that growth chamber studies and small scale field tests with *S. lydicus* strain WYEC 108 have been carried out. Although the studies were not submitted for review, the registrant reported that no deleterious effects were observed. There were no cases of phytotoxicity reported in any of the thirteen efficacy trials conducted with Actinovate SP on strawberry, Gerber daisy and Bell pepper (see Section 5.2). Therefore, while there is reasonable certainty that no deleterious effects are anticipated on nontarget plants, the registrant is required to submit the growth chamber studies and small scale field tests to the PMRA for review to ensure that the MPCA is not pathogenic to potato.

Based on a review of existing scientific information and literature available on the effects of *S. lydicus* to terrestrial organisms, there is reasonable certainty that no harm will be caused to birds, wild mammals, arthropods, non-arthropod invertebrates, or to other microorganisms from the proposed use of Actinovate SP on field and greenhouse strawberry, Bell pepper and Gerber daisy. Therefore the PMRA has accepted the request to waive terrestrial non-target organism testing. However, to ensure that the MPCA is not pathogenic to potato, the registrant is required to submit the growth chamber studies and small scale field tests to the PMRA for review as a condition of registration.

4.2.2 Effects on Aquatic Organisms

The only study submitted to address the risks of Actinovate SP to aquatic organisms was an acute toxicity study in the rainbow trout (*Oncorhynchus mykiss*). In the study, rainbow trout were exposed to *S. lydicus* strain WYEC 108 in the water at concentrations ranging from 3.0×10^3 to 6.3×10^4 CFU/mL (mean measured) under static conditions. There were no mortalities and no adverse effects were reported in any of the test fish. It should be recognized that toxic or pathogenic effects often arise after infection and growth of an organism, which could occur beyond the 96-hour exposure scenario in the study. For this reason the appropriate toxicity test for MPCAs would have been a 30-day freshwater fish test. However, given the lack of infectivity demonstrated in the acute pulmonary toxicity/pathogenicity and infectivity study

and in the intravenous infectivity study conducted on the rat (see Section 3.1), the acute toxicity study on trout was considered acceptable. Microbiological assays conducted on test water showed that the MPCA declined in all test solutions over the 96-hour exposure period, suggesting that it is not adapted to survival in fresh water.

Additional information on the potential risks to aquatic organisms from *S. lydicus* was gathered from published literature. There were no cases of disease or adverse effects in fish or other aquatic organisms (including plants) from *S. lydicus*. However, certain other *Streptomyces* species have demonstrated toxicity to certain aquatic invertebrate organisms, such as brine shrimp (Arthropoda, *Artemia* sp.), sea monkeys (Arthropoda, *Artemia salina*) and freshwater snails (Mollusca, *Oncomelania* sp.). The effects were most often related to the production of secondary metabolites. This is not surprising given that *Streptomyces* are well-known for their production of a vast array of biologically active compounds. There is no evidence to suggest that the MPCA produces any of these compounds.

Use of Actinovate SP will be limited to a foliar application to strawberries, peppers and Gerber daisies in the field or commercial greenhouses. As the product is not approved for aerial application, the intended use pattern minimizes direct exposure to non-target aquatic organisms. However, while the product is not intended for direct application to water, spray drift and surface water runoff from treated fields may result in contamination of aquatic ecosystems. In any event, percolation through soil would most likely result in the MPCA settling in soil, where it is naturally found. In addition, despite reports in published literature which provide evidence for the growth and survival of *Streptomyces* species in water and sediment samples, both as spores and vegetative cells, a rapid decline in the concentration of viable cells in test water in an acute toxicity study with rainbow trout, suggests that the MPCA is not well-adapted to fresh water. Survival and growth in sediment has not been specifically investigated with this species.

The lack of evidence of acute toxicity in rainbow trout in vitro and the absence of reports of disease in aquatic organisms suggests that effects from *S. lydicus* strain WYEC 108 on aquatic organisms are unlikely. However, since other *Streptomyces* species have demonstrated toxicity to aquatic organisms and since effects on aquatic organisms from the MPCA have not been specifically investigated, the possibility that incidents may present themselves in the future cannot be ruled out. Nonetheless, without further testing, given the anticipated terrestrial use pattern of Actinovate SP, there is reasonable certainty that it will be of no harm to aquatic organisms and the requirement for non-target testing on aquatic invertebrates and aquatic plants is waived.

5.0 Value

5.1 Effectiveness Against Pests

5.1.1 Acceptable Efficacy Claims

5.1.1.1 Suppression of Botrytis Fruit Rot (*Botrytis cinerea)* on Field-Grown Strawberry

Two efficacy trials were evaluated for this claim. In one trial on strawberry, Actinovate SP treatments showed a consistent numerical difference between the treated and untreated plants with respect to fruit disease. With Actinovate SP used at the rate of 425 g/1100L water, 40–50% control of disease incidence was noted early in the season. A second trial testing for Botrytis gray mould on rose, also showed a reduction of gray mould (disease incidence). Since no explanation was provided on how disease was assessed, this study can only be considered as supplemental data. However, taken together, there was sufficient evidence to support the claim of suppression of Botrytis fruit rot on strawberries. These data are considered acceptable to extend the use to greenhouse grown strawberries since disease development is the same in both situations.

5.1.1.2 Suppression of Powdery Mildew *(Sphaerotheca macularis)* on Field Grown Strawberry

One trial was evaluated for this claim. Under light disease pressure, Actinovate SP applied at 425 g/1100 L water/ha showed early season suppression (40–60% control) of powdery mildew (disease incidence). It was noted that Actinovate SP performed as well as the standard chemical treatments used by US growers under light disease pressure. However, a consistent level of disease control under high disease pressure could not be confirmed. As a result, a claim of control could not be supported. There was, however, sufficient evidence to support the claim of suppression of powdery mildew on strawberries when applied at 425 g/1100 L water/ha. The data are considered acceptable to extend the use to greenhouse grown strawberries since disease development is the same in both situations.

5.1.1.3 Suppression of Powdery Mildew *(Erysiphe cichoracearum)* on Gerber Daisy grown in the Greenhouse

One trial was evaluated for this claim. Based on the mean number of mildew spots on the plants, Actinovate SP applied at 500 g/1100 L water/ha reduced the level of disease severity compared to the untreated control by 50–60%. The effects were comparable to the commercial standard. Without additional data to demonstrate consistent levels of disease control, the evidence is sufficient to support the claim of suppression only. The data are sufficient to extend the use to field grown Gerber daisy since disease development is the same in both situations.

5.1.1.4 Suppression of Powdery Mildew *(Leveillula taurica)* on Field-Grown Bell Pepper

One trial was evaluated for this claim. Actinovate SP performed as well as typical chemical spray programs used in the US in reducing disease incidence and severity compared to the untreated check (60–90% control) when applied at 425 g/1100 L water/ha. The effects were noted up to three weeks after the final application. However, without additional data to demonstrate consistent levels of control, a claim of suppression only can be supported at this time. The data are sufficient to extend the use to greenhouse peppers since disease development is the same in both situations. This includes all types of pepper since disease is similar on all varieties.

5.1.2 Fungicide Tank Mix Combinations

No data regarding tank-mixing of Actinovate with other registered pest control products were submitted. Compatibility with other products is, therefore, unknown.

5.1.3 Crop Grouping

Little information is known of the biology of *S. lydicus* strain WYEC 108. The rates and effectiveness may change for different ornamentals or agricultural crops. As a result it is not appropriate to consider crop grouping at this time.

5.2 Phytotoxicity to Host Plants

Actinovate SP was tested on three different crops at one rate (425–500 g/ha) applied three to five times per season. No phytotoxicity symptoms were noted in any of the trials for strawberry, Gerber daisy or Bell pepper.

5.3 Impact on Succeeding Crops

No data on impact on succeeding crops were submitted or assessed.

5.4 Economics

No market analysis was submitted for this submission.

5.5 Sustainability

5.5.1 Survey of Alternatives

The registration of Actinovate SP Fungicide will provide a new non-chemical means for managing diseases in strawberry, pepper and Gerber daisy. Several alternatives are available for these uses and are listed in the tables below.

Table 5.5.1.1 Alternative Fungicides/Biopesticides for Control/Suppression of Botrytis Fruit Rot and Powdery Mildew on Strawberry

Technical Grade Active Ingredient	Disease Claim	Fungicide Classification Group
Iprodione	Botrytis Fruit Rot	2
Chlorothalonil	Botrytis Fruit Rot	M
Captan	Gray-Mould Rot	M
Boscalid	Botrytis Gray Mould	7
Vinclozolin	Fruit Rot / Gray Mould	2
Boscalid + Pyraclostrobin	Powdery Mildew	7 and 11

Table 5.5.1.2 Alternative Fungicides/Biopesticides for Control/Suppression of Powdery Mildew on Gerber Daisy

Technical Grade Active Ingredient	Disease Claim	Fungicide Classification Group
Myclobutanil	Powdery Mildew	3

Table 5.5.1.3 Alternative Fungicides/Biopesticides for Control/Suppression of Powdery Mildew on Pepper

Technical Grade Active Ingredient	Disease Claim	Fungicide Classification Group
Myclobutanil	Powdery Mildew	3
Potassium bicarbonate	Powdery Mildew	M

5.5.2 Compatibility with Current Management Practices Including Integrated Pest Management

Actinovate SP Fungicide will fit well with current disease control strategies such as rotation with chemical fungicides. Rotation will also help defer the development of resistance of gray mould and powdery mildew.

5.5.3 Information on the Occurrence or Possible Occurrence of the Development of Resistance

No information is available on the development of pathogen resistance to *S. lydicus* WYEC 108. However, the multiple site mode of action not only creates an effective fungicide but also makes it very difficult for pathogens to develop resistance. Actinovate SP is, therefore, an important tool for growers in areas where resistance to conventional products has developed and it will help to prevent the development of resistant pathogens when it is rotated in a control program with other registered fungicides.

5.5.4 Contribution to Risk Reduction and Sustainability

The registration of Actinovate SP Fungicide will provide a new non-chemical means for managing diseases in strawberry, pepper and Gerber daisy. The multiple site mode of action not only creates an effective fungicide but also makes it very difficult for pathogens to develop resistance. Actinovate SP is therefore an important tool for growers in areas where resistance to conventional products has developed and it will help to prevent the development of resistant pathogens when it is rotated in a control program with other registered fungicides. Actinovate SP Fungicide will fit well with current disease control strategies such as alternation of chemical fungicides.

6.0 Formulants and Microcontaminants of Health or Environmental Concern

6.1 Toxic Substances Management Policy Considerations

The management of toxic substances is guided by the federal government's Toxic Substances Management Policy, which puts forward a preventive and precautionary approach to deal with substances that enter the environment and could harm the environment or human health. The policy provides decision makers with direction and sets out a science-based management framework to ensure that federal programs are consistent with its objectives. One of the key management objectives is virtual elimination from the environment of toxic substances that result predominantly from human activity and that are persistent and bioaccumulative. These substances are referred to in the policy as Track 1 substances.

During the review process *S. lydicus* strain WYEC 108, was assessed in accordance with the PMRA Regulatory Directive DIR99-03, *The Pest Management Regulatory Agency's Strategy for Implementing the Toxic Substances Management Policy*. Substances associated with the use of *S. lydicus* strain WYEC 108, including major transformation products formed in the environment, microcontaminants in the technical product and formulants in the end-use product, Actinovate SP, were also considered. The PMRA has reached the following conclusions:

S. lydicus strain WYEC 108 does not meet the Track-1 criteria because the active ingredient is a biological organism and hence is not subject to the criteria used to define persistence, bioaccumulation and toxicity properties of chemical control products. There are also no

formulants, contaminants or impurities present in the end-use product that would meet the TSMP Track-1 criteria. Therefore, the use of Actinovate SP is not expected to result in the entry of Track-1 substances into the environment.

6.2 Formulants of Health Concern

The technical grade active ingredient, *Streptomyces lydicus* WYEC 108, contains the formulant dairy whey which is identified in Canada Gazette Part II, Volume 139, Number 24, pages 2641–2643: *List of Pest Control Product Formulants of Health or Environmental Concern* as an allergen known to cause anaphylactic-type reactions. Therefore the label for *Streptomyces lydicus* WYEC 108 technical grade active ingredient will include the precautionary statement: “Warning: this product contains the allergen dairy whey” on the principal display panel.

The end-use product, Actinovate SP, contains the formulant dairy whey which is identified in Canada Gazette Part II, Volume 139, Number 24, pages 2641-2643: *List of Pest Control Product Formulants of Health or Environmental Concern* as an allergen known to cause anaphylactic-type reactions. Therefore the label for end-use product, Actinovate SP, will include the precautionary statement: “Warning: this product contains the allergen dairy whey” on the principal display panel.

7.0 Summary

7.1 Methods for Analysis of the Micro-organism as Manufactured

The product characterization data for both the *Streptomyces lydicus* WYEC 108 technical product and Actinovate SP are adequate to assess their risks to human health and the environment. The technical material was fully characterized and the specifications were supported by the analysis of a sufficient number of batches. The quality assurance program is successful at limiting contaminating microorganisms.

Storage stability data were sufficient to support an expiration date of 14 months for *Streptomyces lydicus* WYEC 108 technical product when stored at 21–26°C. Storage stability data for the end-use product was not provided. Therefore, confirmatory storage stability data for Actinovate SP end-use product will be required as a condition of registration. In the interim, a storage interval based on the storage stability data for the Technical grade active ingredient of 12 months at 21–26°C will be specified on the EP label.

7.2 Human Health and Safety

Acute toxicity and infectivity studies (acute oral toxicity, acute pulmonary toxicity/infectivity, intravenous injection infectivity, primary eye irritation and dermal irritation) submitted in support of the *Streptomyces lydicus* WYEC 108 technical product and Actinovate SP were determined to be acceptable. *S. lydicus* strain WYEC 108 was of low toxicity in the rat when administered via the oral route and was not toxic, pathogenic or infective via the pulmonary or intravenous routes. Mild ocular irritation was observed in the eye irritation study. Therefore, the

signal words “CAUTION: EYE IRRITANT” and precautionary statements aimed at instructing users to avoid contact with eyes will be required on the *Streptomyces lydicus* WYEC 108 and Actinovate SP labels. Slight dermal irritation was observed in the acute dermal irritation study.

A waiver request for an acute dermal toxicity/pathogenicity study, based on the results of the submitted toxicity and infectivity studies (including a dermal irritation) and on a comprehensive review of published literature, was considered acceptable to address the potential for dermal toxicity in lieu of testing.

Like all microbial agents, *S. lydicus* is considered a potential sensitizing agent and exposure to *S. lydicus* strain WYEC 108 may cause allergic reactions following repeated exposures at high concentrations. As a result, label statements indicating that *Streptomyces lydicus* WYEC 108 technical and the end-use product Actinovate SP is a potential sensitizer and the requirement for personal protective equipment, including a NIOSH approved respirator and judicious handling to minimize exposure in workers will be required.

When handled according to the label instructions, the potential routes of handler exposure to *S. lydicus* strain WYEC 108 are pulmonary, dermal and to some extent, ocular. Personal protective equipment, judicious handling and a restricted-entry interval of 1 hour will be specified on the *Streptomyces lydicus* WYEC 108 and Actinovate SP labels to minimize exposure in workers.

The label does not allow applications to turf, residential or recreational areas. As the use sites are agricultural, exposure to infants and children in school, residential and daycare facilities is likely to be minimal to non-existent. Consequently, the health risk to infants and children is expected to be negligible.

S. lydicus is a naturally occurring soil microorganism and there have been no adverse effects from dietary exposure attributed to natural populations of *S. lydicus.* Furthermore, there was no significant toxicity observed when *S. lydicus* strain WYEC 108 was administered orally to rats. The establishment of a MRL is therefore not required for *S. lydicus* strain WYEC 108 under Section 4(d) of the *Food and Drugs Act* (adulteration of food) as defined under Division 15, Section B.15.002 of the Food and Drugs Regulations.

7.3 Environmental Risk

Since no studies were submitted to address the risks of Actinovate SP to terrestrial organism, potential risks to terrestrial organisms was assessed based on reports in the published literature, as well as information gathered under Part 3.0 Impact on Human and Animal Health which reported no signs of toxicity or infectivity from *S. lydicus* strain WYEC 108 in laboratory animals. Despite its ubiquitous nature as a common soil microorganism, there were no reports of adverse effects from *S. lydicus* to birds, wild mammals, arthropods (including honeybees) or non-arthropod invertebrates in the published literature. While other *Streptomyces* species have demonstrated toxicity to certain nematode and lepidopteran species by the production of various secondary compounds, there is no indication that *S. lydicus* strain WYEC 108 produces any of these compounds.

Published literature indicated that certain *Streptomyces* species (e.g. *S. scabies*, *S. acidiscabies* and *S. turgidiscabies*) are known to be the causative agents of potato scab. A single report in published literature identified an isolate from a potato scab as *S. lydicus*. Growth chamber studies and small field tests have been carried out with the MPCA and showed no deleterious effects to potato. However, the data was not submitted to the PMRA for review. Published literature indicated that, in general, *S. lydicus* is a natural plant growth promoting bacterium for which beneficial effects have been demonstrated for a range of host plants. In addition, there were no reports of phytotoxicity from the thirteen efficacy trials conducted with Actinovate SP on strawberry, Gerber daisy and Bell pepper. Based on this information, no adverse effects to terrestrial organisms are expected. However, to ensure that no deleterious effects from the MPCA are expected on potato, the growth chamber studies and field tests must be submitted to the PMRA for review as a condition of registration.

The potential risks to aquatic organisms was based on the lack of acute toxicity in rainbow trout exposed to the MPCA in water and on published literature. Published literature did not reveal any reports of adverse effects from *S. lydicus* to aquatic organisms. However, since other *Streptomyces* species have shown toxic effects on aquatic invertebrate organisms and since adverse effects on non-target aquatic organisms from *S. lydicus* have not been specifically investigated, it is possible that effects may present themselves in the future. Furthermore, published literature suggests that *Streptomyces* species may survive in fresh water and marine sediment. However, since the product is not intended for direct application to water, aquatic ecosystems would only be contaminated with Actinovate SP as a result of spray drift and surface water runoff from treated fields. In any event, percolation through soil would most likely result in the MPCA settling in soil where it is naturally found. Furthermore, a decline in concentration of viable cells in water in the rainbow trout study suggests that the MPCA is not well-adapted to survival in fresh water. Based on this assessment, it is not likely that the terrestrial use pattern would result in considerable aquatic exposure and therefore, there is reasonable certainty of no harm to aquatic organisms.

No studies were submitted to address the environmental fate and behaviour of *S. lydicus* strain WYEC 108. Environmental fate data (Tier II/III) are not required due to the absence of significant toxicological effects in non-target organisms in Tier I testing.

7.4 Value

The registration of Actinovate SP would result in an additional non-chemical fungicide for Canadian growers, in particular the greenhouse industry. It could be used as a resistance management tool since the active ingredient has a multiple-site mode of action. Actinovate SP can be used in both in the greenhouse and the field for effective suppression of Botrytis fruit rot on strawberries and powdery mildew on strawberries, Gerber daisies and peppers.

8.0 Proposed Regulatory Decision

Health Canada's PMRA, under the authority of the *Pest Control Products Act*, is proposing full registration for the sale and use of the technical grade active ingredient *Streptomyces lydicus* WYEC 108 and the end-use product Actinovate SP for the suppression of Botrytis fruit rot and powdery mildew on field and greenhouse grown strawberry and powdery mildew on field and greenhouse peppers and Gerber daisies. An evaluation of current scientific data from the applicant, scientific reports and information from other regulatory agencies has resulted in the determination that, under the proposed conditions of use, the end-use product has value and does not present an unacceptable risk to human health or the environment.

List of Abbreviations

ADI	acceptable daily intake
ARfD	acute reference dose
ATCC	American Type Culture Collection
CFU	colony forming units
DNA	deoxyribonucleic acid
EP	end-use product
FDA	*Food and Drugs Act*
H_2S	hydrogen sulfide
KTG	killed test substance group
LC_{50}	lethal concentration 50%
LD_{50}	lethal dose 50%
mL	millilitre
MPCA	microbial pest control agent
MPCP	microbial pest control products
MRL	maximum residue limit
MSH/NIOSH	Mining Safety and Health/National Institute for Occupational Safety and Health
N/A	not applicable
PCPA	*Pest Control Products Act*
PCR	polymerase chain reaction
PPE	personal protective equipment
PMRA	Pest Management Regulatory Agency
RAPD	randomly amplified polymorphic DNA
REI	restricted-entry interval
rRNA	ribosomal ribonucleic acid
TGAI	technical grade of the active ingredient
TSMP	Toxic Substances Management Policy
USEPA	United States Environmental Protection Agency

Appendix I Tables and Figures

Table 1 Toxicity and Infectivity of *Streptomyces lydicus* strain WYEC 108 and Its Associated End-Use Product (Actinovate SP)

Study Type	Species, Strain and Doses	Result	Significant Effects and Comments	Reference
Acute Toxicity/Infectivity of Actinovate SP				
Acute Oral Toxicity	Rat- Sprague Dawley 5/sex treated with WYEC 108-EP[1], 5.4×10^8 CFU/animal	$LD_{50} > 5.4 \times 10^8$ CFU/animal	No major clinical signs indicative of toxicity, no mortalities and no abnormalities on necropsy. Infectivity was not assessed. NON- TOXIC	PMRA 1164642
Acute Pulmonary Toxicity and Infectivity	Rat-Sprague-Dawley TG: 18/sex treated with the MPCA, 9.1×10^8 CFU/animal KTG: 18/sex treated with 9.1×10^8 CFU of heat-killed MPCA/animal Untreated control group (6/sex) and shelf control group (6/sex). Interim sacrifices at Days 3, 7, 14 and 21.	$LC_{50} > 9.1 \times 10^8$ CFU/animal	No major clinical sign indicative of toxicity, no mortalities and no abnormalities on necropsy. The MPCA was detected in the liver and mesenteric lymph nodes until Day 3, in the kidney until Day 7 and in the lungs of treated animals until Day 21, but clearance was established in all organs by Day 28. NON-TOXIC, NOT INFECTIVE	PMRA 1164643
Acute Dermal Toxicity	WAIVER	WAIVER	Based on the lack of observable effects in the primary dermal irritation study conducted with WYEC 108-EP[1], on the lack of toxicity, pathogenicity and infectivity to test animals by the oral, pulmonary and intravenous routes and based on an absence of reports of dermal toxicity frm *S. lydicus* in the published literature, the data waiver request was found to be acceptable to fully assess the risks of dermal toxicity associated with the MPCA in lieu of toxicity testing. WAIVER ACCEPTED	PMRA 1164644

Study Type	Species, Strain and Doses	Result	Significant Effects and Comments	Reference
Intravenous Infectivity	Rat-Sprague-Dawley TG: 18/sex injected with TGAI, 9.33×10^8 CFU/animal KTG: 18/sex injected with 9.33×10^8 CFU of heat-killed TGAI/animal Untreated control group (6/sex) Interim sacrifies at Days 3, 7, 14 and 21.	$LD_{50} > 9.33 \times 10^8$ CFU/animal	No clinical sign indicative of toxicity, no mortalities and no abnormalities on necropsy for the treatment group. The MPCA was detected in the blood, brain, kidney and mesenteric lymph nodes until Day 7, in the lungs until Day 14 and in the liver and spleen until Day 21, but clearance was established in all organs by Day 28. NON-TOXIC, NOT INFECTIVE	PMRA 1164641
Primary Eye Irritation	Rabbit-New Zealand White 0.1g of undiluted WYEC 108-EP[1] (3 animals)	MAS: 0.67/110 (24, 48, 72 hrs.) MIS: 18.67/110 (1 hr.)	Slight opacity, with some swelling occurred at the 1-hour observation. All signs of irritation were resolved by the 72-hour observation. Based on a MIS of 18.67 WYEC 108-EP is classified as "mildly irritating" to the eyes. The signal words "CAUTION: EYE IRRITANT" will be required on the principal display panel of the TGAI and EP label and the precautionary statements "May irritate eyes. Avoid contact with eyes" will be required under the section entitled "Precautions" on the TGAI and EP label.	PMRA 1164649
Dermal Irritation	Rabbit-New Zealand White (3 animals) 0.5g of WYEC 108-EP[1] (5.0×10^8 CFU/g in 0.2 mL of deionized water)	MIS: 0.33/8 (1 hr)	WYEC 108-EP was determined to be "minimally irritating". No signal words are required on the label.	PMRA 1164640

[1] WYEC 108-EP is an alternate formulation of Actinovate SP

TG: Treatment group
KTG: Killed-test substance group
MAS = Mean Average Score; the average score from all animals at the 24-, 48- and 72-hour observation timepoints
MIS = Maximum Irritation Score; the highest average score of all test animals at a single specified time point "

Table 2 Toxicity to Non-Target Species

Organism	Exposure	Test Substance	End Point Value	Significant Effects, Comments	Reference
Terrestrial Organisms					
Terrestrial Vertebrates					
Birds	Oral	A waiver request was submitted in lieu of toxicity testing. The waiver request was based on the natural occurrence of *S. lydicus* in soil, the lack of toxicity and infectivity in test animals as discussed under Part 3[2], the anticipated exposure which is not expected to significantly increase above natural levels of *S. lydicus* in soil and on the results from a literature search which showed no reports of adverse effects in wild bird populations despite the ubiquitous nature of the organism. WAIVER ACCEPTED.			PMRA 1164644[1]
	Pulmonary, inhalation or injection	Based on the waiver request for avian oral toxicity testing and on the lack of toxicity, pathogenicity and infectivity in the acute pulmonary toxicity/infectivity and intravenous injection infectivity studies (Part 3.0[2]), no additional data are required.			Not required
Wild Mammals	Based on information gathered under Part 3.0[2] no additional toxicity testing on wild mammals is required.				Not required
Terrestrial Invertebrates					
Terrestrial Arthropods (including honeybees)	Dietary	A waiver request was submitted in lieu of toxicity testing. The waiver request was based on the natural occurrence of *S. lydicus* in soil, the lack of toxicity and infectivity in test animals (as reported under Part 3.0[2]), the anticipated exposure which is not expected to significantly increase above natural levels of *S. lydicus* in soil and on the results from a literature search which showed no reports of adverse effects to insects, including honeybees, despite the ubiquitous nature of the organism. While other *Streptomyces* species have demonstrated toxicity to certain lepidopteran insect species by the production of biologically active secondary metabolites, there is no indication that *S. lydicus* strain WYEC 108 produces any of these insecticidal compounds. Furthermore, despite the ubiquitous nature of *S. lydicus* strain WYEC 108 as a naturally occurring soil microorganism, there are no reports of insecticidal activity from *S. lydicus* strains in published literature. WAIVER ACCEPTED.			PMRA 1164644[1]

Organism	Exposure	Test Substance	End Point Value	Significant Effects, Comments	Reference
Terrestrial non-arthropod invertebrates (i.e. earthworms)	Acute	Test data are not required as the product is not intended to control pest nonarthropod invertebrates or soil macroorganisms and because the proposed use patterns are not likely to considerably increase natural populations of *S. lydicus* in soil. While other *Streptomyces* species have demonstrated nematocidal activity related to the production of biologically active secondary metabolites, there is no indication that *S. lydicus* strain WYEC 108 produces any of these nematocidal compounds. Furthermore, despite the ubiquitous nature of *S. lydicus* strain WYEC 108 as a naturally occurring soil microorganism, there are no reports of nematocidal activity from *S. lydicus* strains in published literature. No additional information is required.			Not required
Microorganisms	Acute	Test data are not required as *S. lydicus* strain WYEC 108 is a naturally occurring soil microorganism and the organism is not expected to affect environmentally or economically important microbial species or microbiologically-mediated biogeochemical processes. No additional information is required.			Not required
Terrestrial Plants					
Terrestrial Plants	Acute	A waiver request was submitted in lieu of toxicity testing. The waiver request was based on the natural occurrence of *S. lydicus* in soil as a plant growth promoting bacterium, the anticipated exposure which is not expected to significantly increase above natural levels of *S. lydicus* in soil and the lack of phytotoxicity was observed in field trials. Although one report in published literature isolated a *S. lydicus* strain from a potato scab, literature validated that this species is not usually associated with potato scab disease and the MPCA was shown to be non-phytotoxic to potatoes in growth chamber and field studies. WAIVER ACCEPTED. To ensure that no deleterious effects from the S. lydicus strain WYEC 108 are expected on potato, the PMRA will require that the growth chamber study and small-scale field tests be submitted for review.			PMRA 1164644[1]

Organism	Exposure	Test Substance	End Point Value	Significant Effects, Comments	Reference
Aquatic Organisms					
Aquatic Vertebrates					
Freshwater fish	Acute	In a 96-hour acute toxicity study, there were no mortalities and no adverse effects in rainbow trout exposed to *S. lydicus* strain WYEC 108 in the water at concentrations ranging from 1.5×10^4 to 6.3×10^4 CFU/mL. $LC_{50} > 6.3 \times 10^4$ CFU/mL			PMRA 1164653
Estuarine/ marine fish	Acute	Test data is not required as estuarine and marine fish are not expected to be exposed to the MPCA.			Not required
Aquatic Invertebrates					
Aquatic arthropods (Aquatic invertebrates)	Acute	A waiver request was submitted in lieu of toxicity testing. The waiver request was based on the lack of toxicity and infectivity in test animals (as reported under Part 3.0[2]), the lack of acute toxicity in freshwater fish in vitro and the minimal anticipated exposure to aquatic environments. While certain *Streptomyces* species have demonstrated toxicity to brine shrimp (Arthropoda, *Artemia* sp.) by the production of biologically active secondary metabolites, there is no indication that *S. lydicus* strain WYEC 108 produces these compounds. Furthermore, there are no reports of toxicity to aquatic arthropod invertebrates from *S. lydicus* strains in published literature. WAIVER ACCEPTED.			PMRA 1164644[1]
Aquatic non-arthropod invertebrates (i.e. molluscs)	Acute	Based on the lack of toxicity and infectivity in test animals (as reported under Part 3.0[2]), the lack of acute toxicity in freshwater fish in vitro and on the minimal anticipated exposure to aquatic environments, no additional data is required to assess the effects of *S. lydicus* strain WYEC 108 on aquatic non-arthropod invertebrates. While certain *Streptomyces* species have demonstrated toxicity to freshwater snails (Mollusca, *Oncomelania* sp.) by the production of biologically active secondary metabolites, there is no indication that *S. lydicus* strain WYEC 108 produces these compounds. Furthermore, there are no reports of toxicity to aquatic non-arthropod invertebrates from *S. lydicus* strains in published literature. No additional information is required.			Not required

Organism	Exposure	Test Substance	End Point Value	Significant Effects, Comments	Reference
Aquatic Plants					
Aquatic plants	Acute	Based on the low anticipated exposure to aquatic environments and the lack of reports of adverse effects from *S. lydicus* to aquatic plants in published literature, no additional information is required.			Not required

[1] A single report, addressing the data waiver request for the outstanding environmental toxicity studies, was submitted.

[2] Information submitted in Part 3.0 (Impact on Human and Animal Health) includes an acute oral toxicity study, an acute pulmonary toxicity/pathogenicity and infectivity study, an intravenous injection infectivity study, a dermal irritation study and an eye irritation study.

References

A. List of Studies Submitted by Registrant

1.0 The Active Ingredient Its Properties and Uses

PMRA Number	Reference
PMRA 1164650	Product chemistry of Actinovate SP. Project No. 04-059. April 24, 2000. 28 pages. DACO 2.1-2.11
PMRA 1360736	University of Idaho. 1998. Thesis: Mechanism of biocontrol of fungal root pathogens in the rhizosphere by *Streptomyces lydicus* WYEC 108. Ph. D. Microbiology. Moscow, ID. 81 pages.
PMRA 1360737	University of Idaho. 2002. Plant growth promotion and siderophore production by selected root-colonizing nonpathogenic *Streptomyces* species. M. Sc.
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PMRA 1348801	Locci, R. 1989. *Streptomycetes* and related genera. In: Bergey's Manual of Systematic Bacteriology. Vol. 4. 2451-2492.
PMRA 1164646	Biopesticide Regulatory Action Document. *Streptomyces lydicus* WYEC 108. USEPA. April 27, 2004. 41 pages.

2.0 Methods of Analysis

PMRA Number	Reference
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PMRA 1348800	Response to Clarifax. Dec. 20, 2000. 5 pages.
PMRA 1360731	Natural Industries Inc.: Summary of storage stability information. Jan. 18, 2007.
PMRA 1348805	Summary of physical and chemical properties: Actinovate SP (EP) and *Streptomyces lydicus* WYEC 108 (TGAI). 1 page.
PMRA 1360734	Goodfellow, M. And K. E. Simpson. 1987. Ecology of Streptomycetes. Frontiers Applied Microbiology. 2: 97-125.

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PMRA 1164649 Final Report: Primary Eye Irritation Study in the Rabbit. Laboratory Study No. 5200-99. September 2, 1999. 16 pages. Unpublished.

PMRA 1164640 Final Report: Acute Dermal Irritation Study in Rabbit Laboratory Study No. 5201-99. September 2, 2000. 13 pages. Unpublished.

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4.0 Impact on the Environment

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B. Additional Information Considered

i) Published Information

1.0 The Active Ingredient, Its Properties and Uses

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